

FIG. 1A**Nucleotide sequence of EOS (SEQ.ID.NO1)**

CCACGCGTCCGACCAGAGTCCAAGCCCTAGGCAGTGCCACCCTTACCCAGCCCAGCCTTG
AAGACAGAATGAGAGGGGTTTCCTGTCTCCAGGTCTGCTCCTTCTGGTGCTGGGAGCTG
CTGGGACTCAGGGAAGGAAGTCTGCAGCCTGCGGGCAGCCCCGCATGTCCAGTCGGATCG
TTGGGGGCCGGGATGGCCGGGACGGAGAGTGGCCGTGGCAGGCGAGCATCCAGCATCCTG
GGGCACACGTGTGCGGGGGGTGCTCATGCCCCCAGTGGGTGCTGACAGCGGGCGCACT
GCTTCCCCAGGAGGGCACTGCCAGCTGAGTACCGCGTGCGCCTGGGGGCGCTGCGTCTGG
GCTCCACCTCGCCCCGCACGCTCTCGGTGCCCCGTGCGACGGGTGCTGCTGCCCCCGGACT
ACTCCGAGGACGGGGCCCCGCGCGACCTGGCACTGCTGCAGCTGCGTCCGCCGGTGCCCC
TGAGCGCTCGCGTCCAACCCGTCTGCCTGCCCCGTGCCCCGCGCCCCGCCGCCCGGCA
CACCATGCCGGGTACCCGGCTGGGGCAGCCTCCGCCCAGGAGTGCCCCCTCCCAGAGTGGC
GACCGCTACAAGGAGTAAGGGTGCCGCTGCTGGACTCGCGCACCTGCGACGGCCTCTACC
ACGTGGGCGCGGACGTGCCCCAGGCTGAGCGCATTGTGCTGCCTGGGAGTCTGTGTGCCG
GCTACCCCCAGGGCCACAAGGACGCTGCCAGGGTGATTCTGGGGGACCTCTGACCTGCC
TGCAGTCTGGGAGCTGGGTCTTGGTGGGCGTGGTGAGCTGGGGCAAGGGTTGTGCCCTGC
CCAACCGTCCAGGGGTCTACACCAGTGTGGCCACATATAGCCCCCTGGATTTCAGGCTCGCG
TCACTTCTAATGCTAGCCGGTGAGGCTGACCTGGAGCCAGCTGCTGGGGTCCCTCAGCCT
CCTGGTTTCATCCAGGCACCTGCCTATACCCACATCCCTTCTGCCTCGAGGCCAAGATGC
CTAAAAAAGCTAAAGGCCACCCACCCCCACCCACCACCTTCTGGCTCCTCTCCTCTTT
GGGGATCACCAGCTCTGACTCCACCAACCCTCATCCAGGAATCTGCCATGAGTCCCAGGG
AGTCACACTCCCCACTCCCTTCCTGGCTTGTATTACTTTTCTTGGCCCTGGCCAGGGCT
GGGCGCAAGGCACGCAGTGATGGGCAAACCAATTGCTGCCCATCTGGCCTGTGTGCCCAT
CTTTTTCTGGAGAAAGTCAGATTTCACAGCATGACAGAGATTTGACACCAGGGAGATCCTC
CATAGCTGGCTTTGAGGACACGGGGACCACAGCCATGAGCGGCCTCTAAGAGCTGAGAGA
CAGCCGGCAGGGAATCGGAACCCTCAGACCCACAGCCGCAAGGCACTGGATTCTGGCAGC
ACCCTGAAGGAGCTGGGAAGTAAGTTCTTCCCCAGCCTCCAGATAAGAGCCCCGCCGGCC
AATCCCTTCATTTCAACCTAAAGAGACCCTAAGCAGAGAACCTAGCTGAGCCACTCCTGA
CCTACAAAGTTGTGACTTAATAAATGTGTGCTTTAAGCTGCCAAAAAAAAAAAA

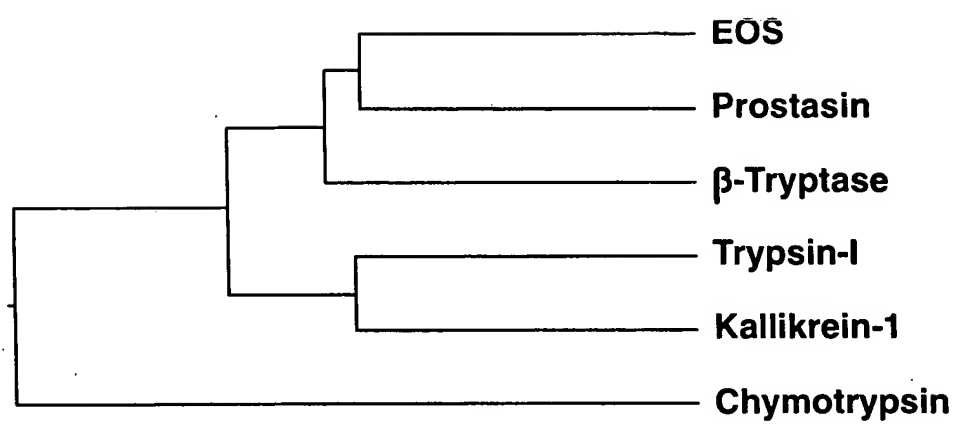
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FIG. 1B**Amino Acid sequence of EOS protease (SEQ.ID.NO.:7)**

Met	Arg	Gly	Val	Ser	Cys	Leu	Gln	Val	Leu	Leu	Leu
Leu	Val	Leu	Gly	Ala	Ala	Gly	Thr	Gln	Gly	Arg	Lys
Ser	Ala	Ala	Cys	Gly	Gln	Pro	Arg	Met	Ser	Ser	Arg
Ile	Val	Gly	Gly	Arg	Asp	Gly	Arg	Asp	Gly	Glu	Trp
Pro	Trp	Gln	Ala	Ser	Ile	Gln	His	Pro	Gly	Ala	His
Val	Cys	Gly	Gly	Ser	Leu	Ile	Ala	Pro	Gln	Trp	Val
Leu	Thr	Ala	Ala	His	Cys	Phe	Pro	Arg	Arg	Ala	Leu
Pro	Ala	Glu	Tyr	Arg	Val	Arg	Leu	Gly	Ala	Leu	Arg
Leu	Gly	Ser	Thr	Ser	Pro	Arg	Thr	Leu	Ser	Val	Pro
Val	Arg	Arg	Val	Leu	Leu	Pro	Pro	Asp	Tyr	Ser	Glu
Asp	Gly	Ala	Arg	Gly	Asp	Leu	Ala	Leu	Leu	Gln	Leu
Arg	Arg	Pro	Val	Pro	Leu	Ser	Ala	Arg	Val	Gln	Pro
Val	Cys	Leu	Pro	Val	Pro	Gly	Ala	Arg	Pro	Pro	Pro
Gly	Thr	Pro	Cys	Arg	Val	Thr	Gly	Trp	Gly	Ser	Leu
Arg	Pro	Gly	Val	Pro	Leu	Pro	Glu	Trp	Arg	Pro	Leu
Gln	Gly	Val	Arg	Val	Pro	Leu	Leu	Asp	Ser	Arg	Thr
Cys	Asp	Gly	Leu	Tyr	His	Val	Gly	Ala	Asp	Val	Pro
Gln	Ala	Glu	Arg	Ile	Val	Leu	Pro	Gly	Ser	Leu	Cys
Ala	Gly	Tyr	Pro	Gln	Gly	His	Lys	Asp	Ala	Cys	Gln
Gly	Asp	Ser	Gly	Gly	Pro	Leu	Thr	Cys	Leu	Gln	Ser
Gly	Ser	Trp	Val	Leu	Val	Gly	Val	Val	Ser	Trp	Gly
Lys	Gly	Cys	Ala	Leu	Pro	Asn	Arg	Pro	Gly	Val	Tyr
Thr	Ser	Val	Ala	Thr	Tyr	Ser	Pro	Trp	Ile	Gln	Ala
Arg	Val	Thr	Ser	Asn	Ala	Ser	Arg				

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FIG. 2



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FIG. 3

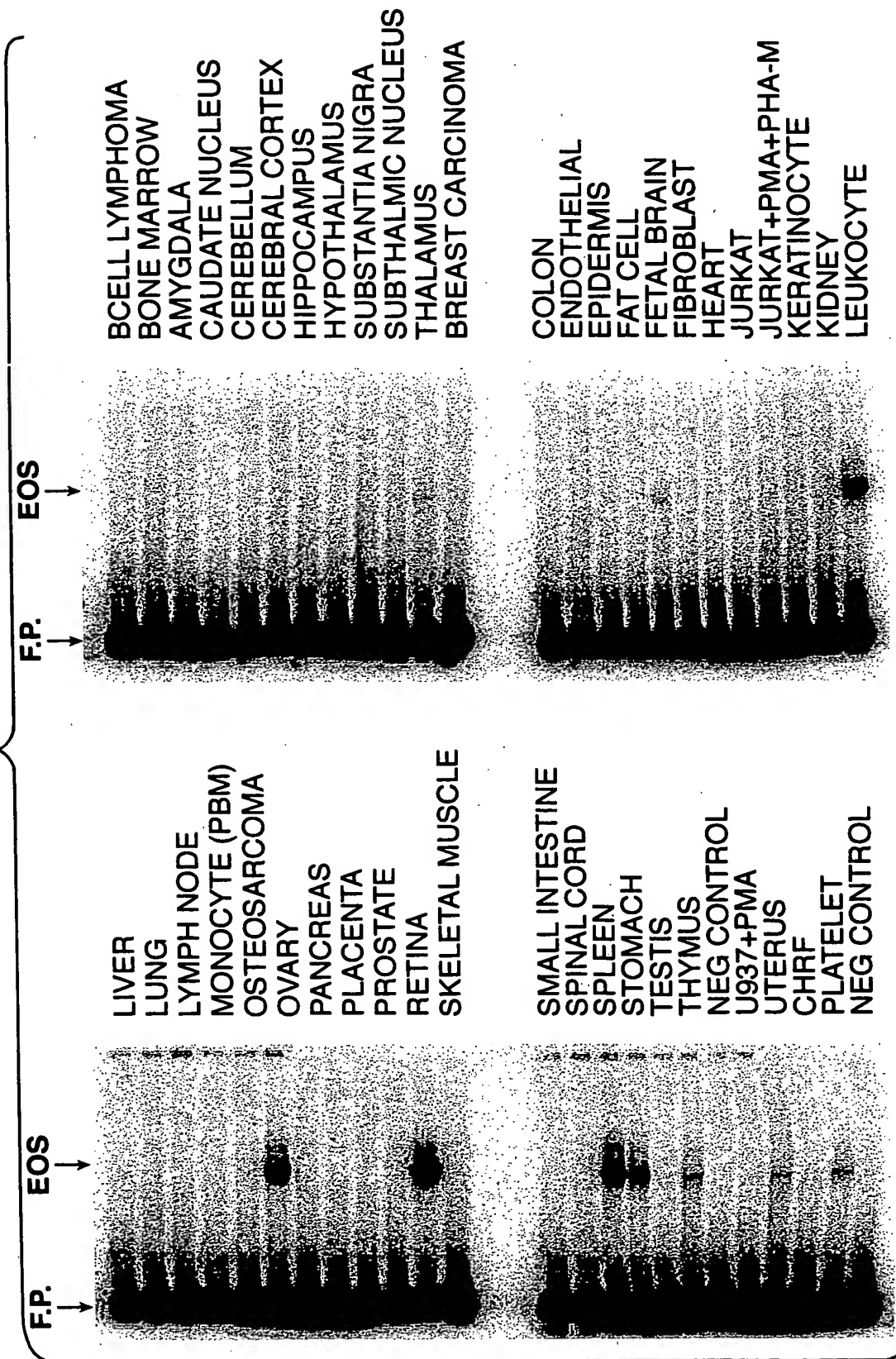


FIG. 4AEco RI

GAATTCACCACCATGGACAGCAAAGGTTCTGTCGCAGAAATCCCGCCTGCT
 1 -----+-----+-----+-----+-----+ 50
 CTTAAGTGGTGGTACCTGTCGTTTCCAAGCAGCGTCTTTAGGGCGGACGA
 M D S K G S S Q K S R L L
 Prolactin Signal Sequence

CCTGCTGCTGGTGGTGTCAAATCTACTCTTGTGCCAGGGTGTGGTCTCCG
 51 -----+-----+-----+-----+-----+ 100
 GGACGACGACCACCACAGTTTAGATGAGAACACGGTCCCACACCAGAGGC
 L L L V V S N L L L C Q G V V S
 Prolactin Signal Sequence

Not I

ACTACAAGGACGACGACGACGTGGACGCGGCCGCTCTTGCTGCCCCCTTT
 101 -----+-----+-----+-----+-----+ 150
 TGATGTTCTGCTGCTGCTGCACCTGCGCCGGCGAGAACGACGGGGGAAA
 D Y K D D D D V D A A A L A A P F
 FLAG EK Pro

Xba I

GATGATGATGACAAGATCGTTGGGGGCTATGCTCTAGAGGACGGAGAGTG
 151 -----+-----+-----+-----+-----+ 200
 CTACTACTACTGTTCTAGCAACCCCGATACGAGATCTCCTGCCTCTCAC
 D D D D K I V G G Y A L E D G E W
 EK Pro

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FIG. 4B

201 GCGTGGCAGGCGAGCATCCAGCATCCTGGGGCACACGTGTGCGGGGGGT 250
-----+-----+-----+-----+-----+
CGGCACCGTCCGCTCGTAGGTCGTAGGACCCCGTGTGCACACGCCCCCA
P W Q A S I Q H P G A H V C G G
----- Protease EOS.CDS -----

251 CGCTCATCGCCCCCAGTGGGTGCTGACAGCGGCGCACTGCTTCCCCAGG 300
-----+-----+-----+-----+-----+
GCGAGTAGCGGGGGGTCACCCACGACTGTGCGCCGCGTGACGAAGGGGTCC
S L I A P Q W V L T A A H C F P R
----- Protease EOS.CDS -----

301 AGGGCACTGCCAGCTGAGTACCGCGTGCGCCTGGGGGCGCTGCGTCTGGG 350
-----+-----+-----+-----+-----+
TCCCGTGACGGTCGACTCATGGCGCACGCGGACCCCGCGACGCAGACCC
R A L P A E Y R V R L G A L R L G
----- Protease EOS.CDS -----

351 CTCCACCTCGCCCCGCACGCTCTCGGTGCCC GTGCGACGGGTGCTGCTGC 400
-----+-----+-----+-----+-----+
GAGGTGGAGCGGGGCGTGCGAGAGCCACGGGCACGCTGCCACGACGACG
S T S P R T L S V P V R R V L L
----- Protease EOS.CDS -----

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FIG. 4C

401 C C C C G G A C T A C C G A G G A C G G G G C C C G C G G C G A C C T G G C A C T G C T G C A G
-----+-----+-----+-----+ 450
G G G G C C T G A T G A G G C T C C T G C C C G G G C G C C G C T G G A C C G T G A C G A C G T C
P P D Y S E D G A R G D L A L L Q

Protease EOS.CDS

451 C T G C G T C G C C C G G T G C C C C T G A G C G C T C G C G T C C A A C C C G T C T G C C T G C C
-----+-----+-----+-----+ 500
G A C G C A G C G G G C C A C G G G G A C T C G C G A G C G C A G G T T G G G C A G A C G G A C G G
L R R P V P L S A R V Q P V C L P

Protease EOS.CDS

501 C G T G C C C G G C G C C C G C C C G C C C G G C A C A C C A T G C C G G G T C A C C G G C T
-----+-----+-----+-----+ 550
G C A C G G G C C G C G G G C G G G C G G C G G G C C G T G T G G T A C G G C C C A G T G G C C G A
V P G A R P P P G T P C R V T G

Protease EOS.CDS

551 G G G G C A G C C T C C G C C C A G G A G T G C C C C T C C C A G A G T G G C G A C C G C T A C A A
-----+-----+-----+-----+ 600
C C C G T C G G A G G C G G G T C C T C A C G G G A G G G T C T C A C C G C T G G C G A T G T T
W G S L R P G V P L P E W R P L Q

Protease EOS.CDS

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FIG. 4D

601 GGAGTAAGGGTGCCGCTGCTGGACTCGCGCACCTGCGACGGCCTCTACCA 650
-----+-----+-----+-----+
CCTCATTCCCACGGCGACGACCTGAGCGCGTGGACGCTGCCGGAGATGGT
G V R V P L L D S R T C D G L Y H

Protease EOS.CDS

651 CGTGGGCGCGGACGTGCCCCAGGCTGAGCGCATTGTGCTGCCTGGGAGTC 700
-----+-----+-----+-----+
GCACCCGCGCCTGCACGGGGTCCGACTCGCGTAACACGACGGACCCTCAG
V G A D V P Q A E R I V L P G S

Protease EOS.CDS

701 TGTGTGCCGGCTACCCCCAGGGCCACAAGGACGCCTGCCAGGGTGATTCT 750
-----+-----+-----+-----+
ACACACGGCCGATGGGGGTCCCGGTGTTCTGCGGACGGTCCCCTAAGA
L C A G Y P Q G H K D A C Q G D S

Protease EOS.CDS

751 GGGGGACCTCTGACCTGCCTGCAGTCTGGGAGCTGGGTCCTGGTGGGCGT 800
-----+-----+-----+-----+
CCCCCTGGAGACTGGACGGACGTCAGACCCTCGACCCAGGACCACCCGCA
G G P L T C L Q S G S W V L V G V

Protease EOS.CDS

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FIG. 4E

801 GGTGAGCTGGGGCAAGGGTTGTGCCCTGCCCAACCGTCCAGGGGTCTACA
-----+-----+-----+-----+-----+ 850
CCACTCGACCCCGTTCCCAACACGGGACGGGTGGCAGGTCCCCAGATGT
V S W G K G C A L P N R P G V Y

Protease EOS.CDS

851 CCAGTGTGGCCACATATAGCCCCTGGATTCAGGCTCGCGTCACTTCTAAT
-----+-----+-----+-----+-----+ 900
GGTCACACCGGTGTATATCGGGGACCTAAGTCCGAGCGCAGTGAAGATTA
T S V A T Y S P W I Q A R V T S N

Protease EOS.CDS

Xba I
901 GCTTCTAGATACCCCTACGATGTGCCCCGATTACGCCGCTAGACATCACCA
-----+-----+-----+-----+-----+ 950
CGAAGATCTATGGGGATGCTACACGGGCTAATGCGGCGATCTGTAGTGGT
A S R Y P Y D V P D Y A A R H H H

HA/HIS-TAG

Not I
951 TCACCATCACTAGCGGCCGCTTCCCTTTAGTGAGGGTTAATGCTTCGAGC
-----+-----+-----+-----+-----+ 1000
AGTGGTAGTGATCGCCGGCGAAGGGAAATCACTCCCAATTACGAAGCTCG
H H H *

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FIG. 4F

1001 AGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGC 1050
-----+-----+-----+-----+-----+
TCTGTACTATTCTATGTAATACTCAAACCTGTTTGGTGTGATCTTACG

SV40 Late pA

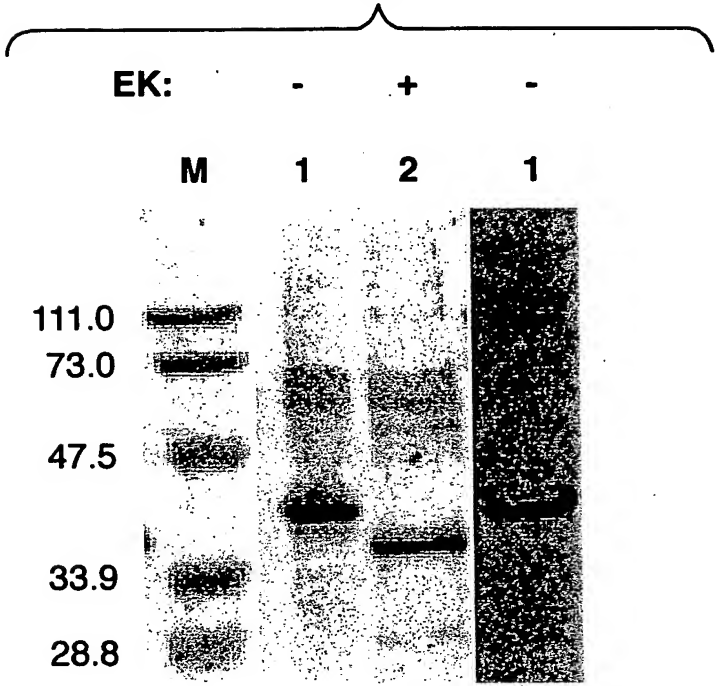
1051 AGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTT 1100
-----+-----+-----+-----+-----+
TCACTTTTTTTACGAAATAAACACTTTAAACACTACGATAACGAAATAAA

SV40 Late pA

1101 GTAACCATTATAAGCTGCAATAAACAAGTT 1130
-----+-----+-----+
CATTGGTAATATTTCGACGTTATTTGTTCAA

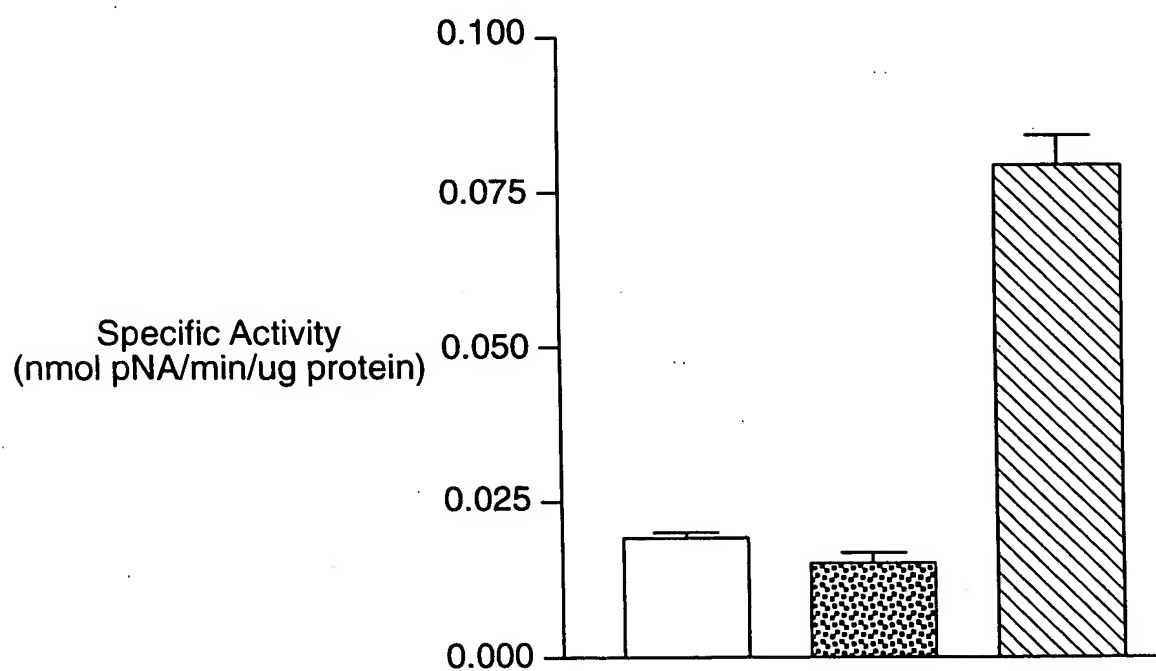
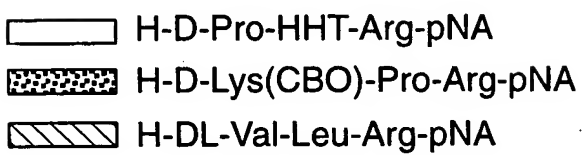
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FIG. 5

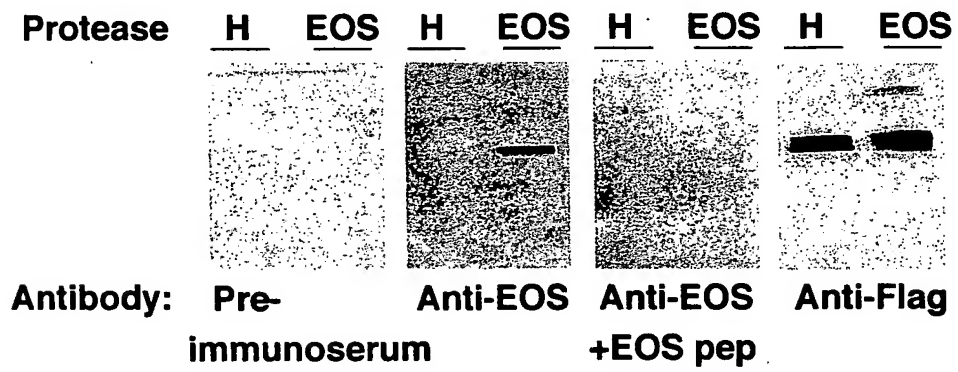


Protease: PFEK2-EOS-6XHIS

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FIG. 6**Protease Activity of EOS**

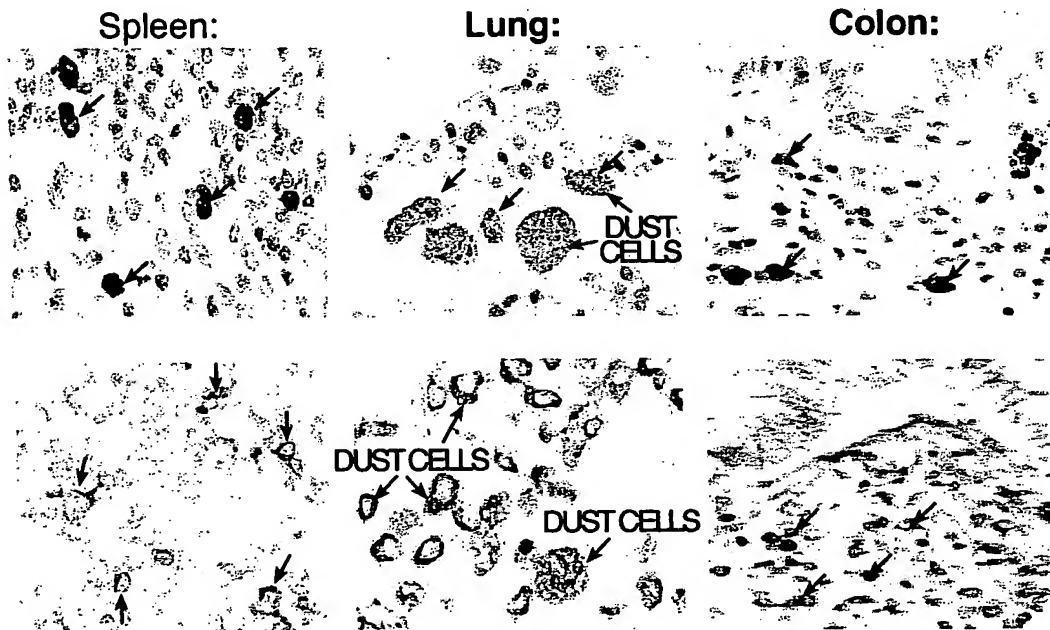
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FIG. 7**Anti-EOS Antiserum Immunoblot Characterization**

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FIG. 8

Localization of EOS protein (top) and mRNA (bottom)
in human spleen, lung, and colon



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FIG. 9

Double immunofluorescence (IF:IF) of
EOS and macrophage marker:CD68



EOS in human colon



CD68 in human colon

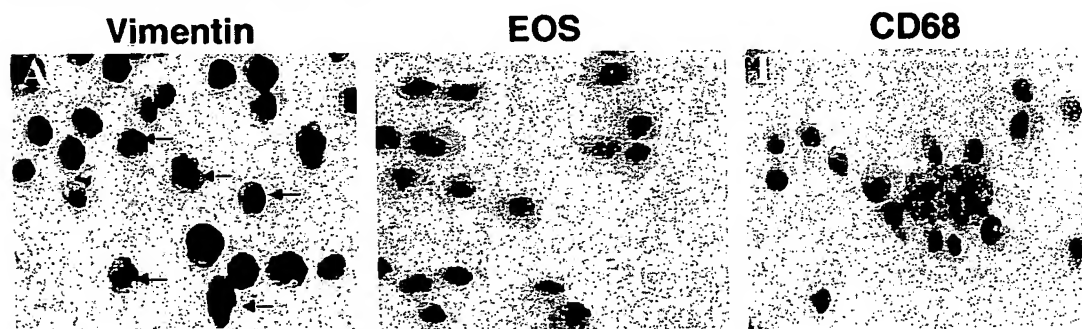
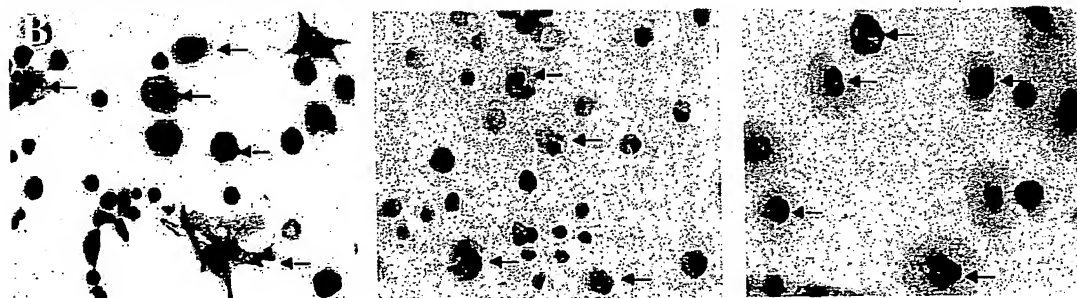
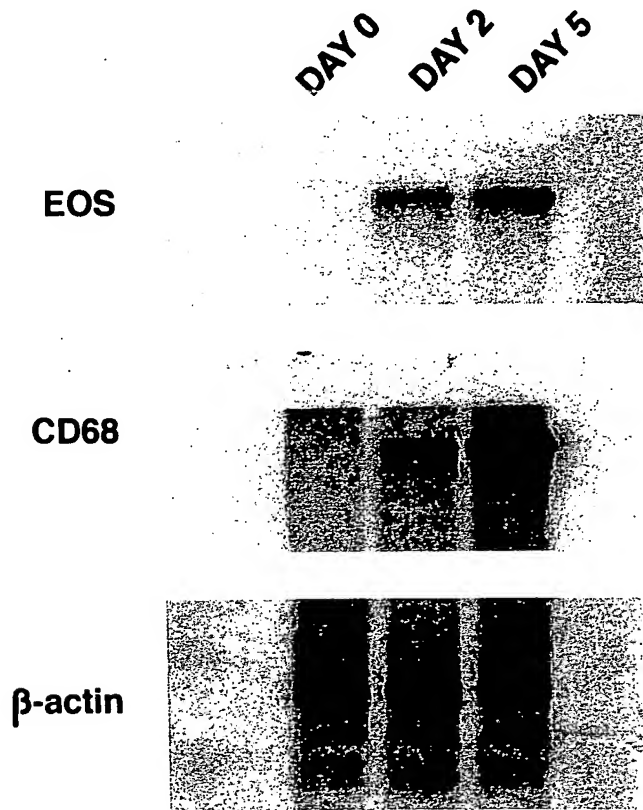
FIG. 10**Up-regulation of EOS protein by PMA in U937 cells****Untreated U937 cells:****PMA treated U937 cells:**

FIG. 11

Up-regulation of EOS mRNA by PMA in U937 cells



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